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# Effect of the Extract of *Fructus Tribuli* on Growth of *Lactobacillus Acidophilus*

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**Abstract**

Effect of the extract of *Fructus tribuli* (EFT) on growth of Lactobacillus acidophilus LA04, LA05, LA06 and LA09 was studied by measuring optical density at 600nm (OD600) and pH using MRS media as the control. The addition of EFT (v/v) was 1%, 2%, 3%, 4% and 5%. Results were as follows: addition of EFT could promote the growth of Lactobacillus acidophilus LA09, The optimum concentration of EFT in MRS media was 1% at incubation 24h, but addition of EFT on growth of Lactobacillus acidophilus LA04, LA05 and LA06 has no significant effect.



*Keywords:*Lactobacillus acidophilus; probiotics; Fructus tribuli;

## 1. Introduction

The probiotics are "Live microorganisms, which when administered in adequate amounts confer a health benefit on the host". The probiotics that have been traditionally most often used are the lactic bacteria of the genera Lactobacillus, Streptococcus, the Bifidobacterium plus *Saccharomyces boulardii*, which have been introduced in food products and drugs due to their growing evidence of health benefits [1-2]. Strains of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* strain GG probably have the longest history of application as probiotics. Clinical studies have also supported the role of probiotics in treating pouchitis, antibioticassociated diarrhea (AAD) and travelers' diarrhea, Crohn's disease, ulcerative colitis (UC), irritable bowel syndrome (IBS), Helicobacter pylori infections, lactose intolerance, immunomodulation, lowering of blood cholesterol [3-8].

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*Tribulus terrestris* is an annual herb that grows all over the world [9].The fruit, stems and leaves of the plant are used in the folk medicine in China against various diseases, including impotence tonic, cardiovascular diseases, anti-hypertensive urinary anti-infective, anti-inammatory and immuno-suppressive activities [10-11].Fructus tribuli is the fruit of Tribulus terrestris and allowed to use in functional food by the Ministry of Health of China. Furostanol and spirostanol saponins, polysaccharides, flavonoid glycosides, alkaloids, alkaloids and some amides have been reported to occur in Fructus Tribuli [12-15].

In previous research, we found that the hot water extract from Fructus tribuli can promote the growth of L.acidophilus acidophilus WS [16]. The purpose of the present work was to study effect of EFT on growth of Lactobacillus acidophilus LA04, LA05, LA06 and LA09.

## 2. Materials and methods

### *2.1. Microorganism*

*Lactobacillus acidophilus* LA04, LA05, LA06 and LA09 were obtained from College of Life Science & Engineering, Shaanxi University of Science & Technology. All lactobacilli strains were grown three successive times in MRS medium (Hopebio, Qingdao, China) before use.

### *2.2. Preparation of the extract from Fructus tribuli*

Dried *Fructus tribuli* were purchased from a local herb market (Xi'an, China), Fructus tribuli was ground in a high speed disintegrator (Model SF-2000, Shanghai, China) to obtain a fine powder, then was extracted distilled water (liquid: solid ratio (ml/g) 14:1, While the temperature of the water bath was kept steady, The extraction in a 1.0 l stainless steel boiler in the water bath was stirred with an electric mixing paddle for 1.5h, then obtained the extract of Fructus tribuli (EFT) by filtration.

### *2.3. Culture conditions*

EFT was added to MRS media in anaerobic tube at 1.0%, 2.0%, 3.0%, 4.0% and 5.0% (v/v), respectively. MRS media without EFT was included in this experiment as a control.

All samples were autoclaved at 118℃ for 15 min. Active culture was added to each anaerobic tube after cooling to room temperature and incubated at 37℃, then determined biomass and pH at incubation 12h, 18h and 24h. The experiment was completed in triplicate and means were calculated for each treatment group at each data collection point.

### *2.4. Biomass and pH measurement*

The biomass was determined by optical density at 600nm (OD600) through a spectrophotometer (SP-756PC, Shanghai Spectrum Instruments Co., Ltd., Shanghai, China).The pH of culture was measured through a pH-meter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China).

## 3. Results and discussion

### *3.1. Effect of EFT on growth of L. acidophilus LA04*

Effect of different concentrations of EFT on the growth of *Lactobacillus acidophilus LA04* showed in Figure 1. With the increasing concentration of EFT, OD value increased gradually and reached maximum 1.605, 1.788 and 1.772 at 1%, 4% and 2% EFT, then decreased to 1.542, 1.728 and 1.720 at 5% EFT at incubation 12h, 18h and 24h, respectively. The OD value of the control was 1.574, 1.726 and 1.736, respectively; the pH of different culture mediums has no significant difference ranging from 4.06 to 4.12(incubation time 12h), 3.79-3.84(incubation time 18h) and 3.70-3.76(incubation time 24h), which indicated that EFT on growth *of Lactobacillus acidophilus LA04* has no significant role.

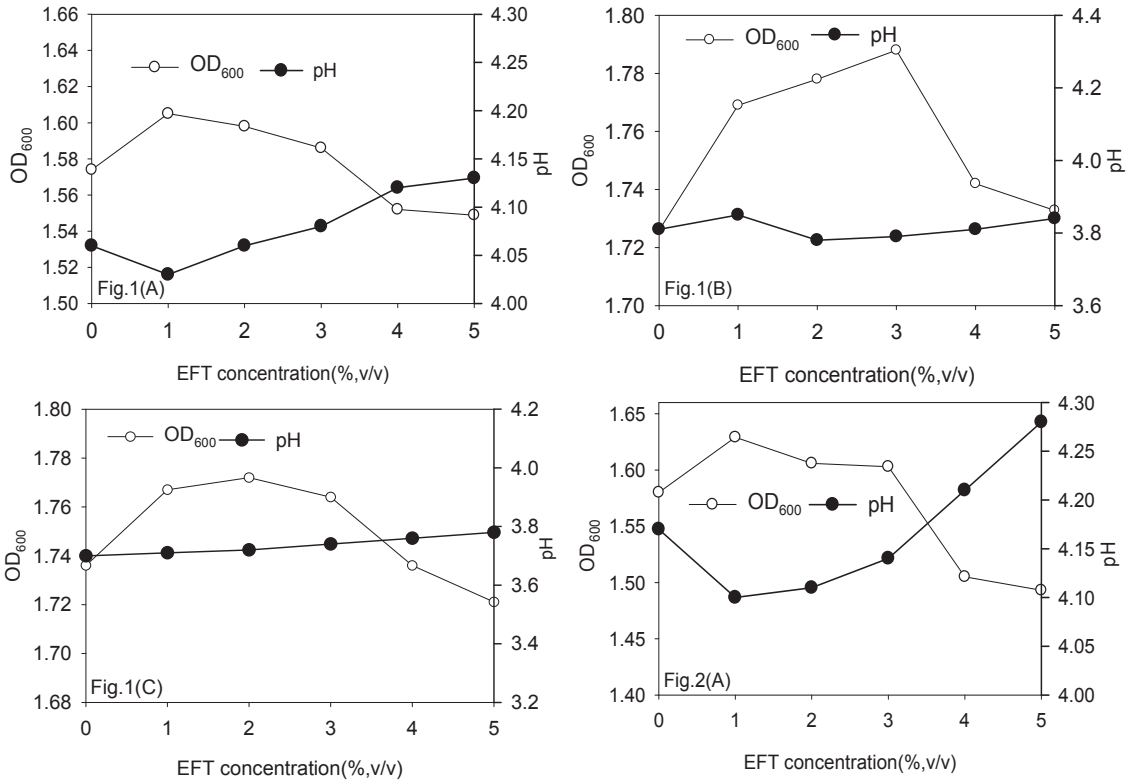



Fig1.Effect of EFT on growth of *Lactobacillus acidophilus LA04*,A,B and C mean incubation 12h,18h and 24h,respectively

### *3.2. Effect of EFT on growth of L. acidophilus LA05*

Effect of different concentrations of EFT on the growth of *Lactobacillus acidophilus LA05* showed in Figure 2. With the increasing concentration of EFT, OD value increased gradually and reached maximum 1.629, 1.797 and 1.783 at 1% EFT, then decreased to 1.493, 1.575 and 1.539 at 5% EFT at incubation 12h, 18h and 24h, respectively. The OD value of the control was 1.580, 1.746 and 1.734, respectively; the pH of different culture mediums in contrast with the $OD_{600}$ has no significant difference ranging from 4.10 to 4.28(incubation time 12h), 3.77-4.12(incubation time 18h) and 3.70-4.04(incubation time 24h), which indicated that EFT on growth *of Lactobacillus acidophilus LA05* has no obvious effect.

### *3.3. Effect of EFT on growth of L. acidophilus LA06*

Effect of different concentrations of EFT on the growth of *Lactobacillus acidophilus LA06* showed in Figure 3. With the increasing concentration of EFT, OD value increased gradually and reached maximum 1.627 and 1.642 at 2% and 1% EFT, then decreased to 1.567 and 1.61 at 5% EFT at incubation 12h and 18h, respectively. The OD value increased gradually from 1.603 to 1.621 at 5% EFT at incubation 24h, The $OD_{600}$ of the control was 1.567, 1.616 and 1.578, respectively; the pH of different culture mediums in contrast with the $OD_{600}$ has no significant difference ranging from 4.10 to 4.28(incubation time 12h), 3.77-4.12(incubation time 18h) and 3.70-4.04(incubation time 24h), which indicated that EFT on growth of *Lactobacillus acidophilus LA06* has no significant role.

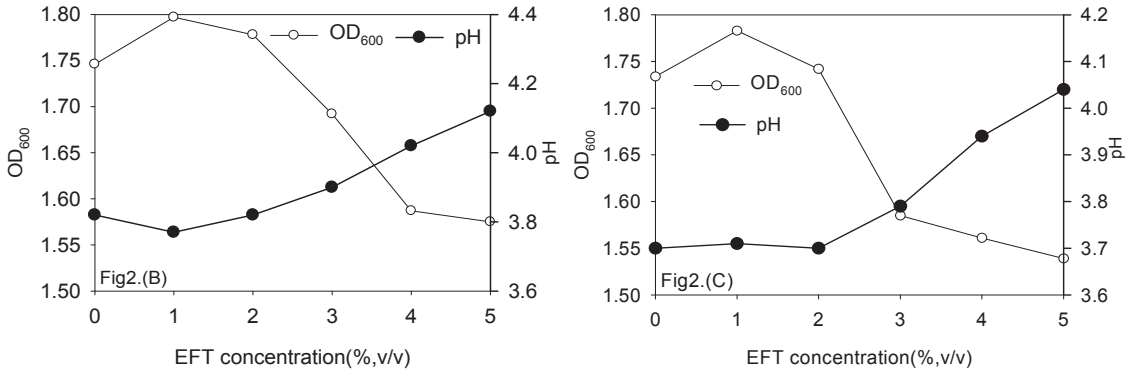



Fig2.Effect of EFT on growth of *Lactobacillus acidophilus LA05*,A,B and C mean incubation 12h,18h and 24h,respectively

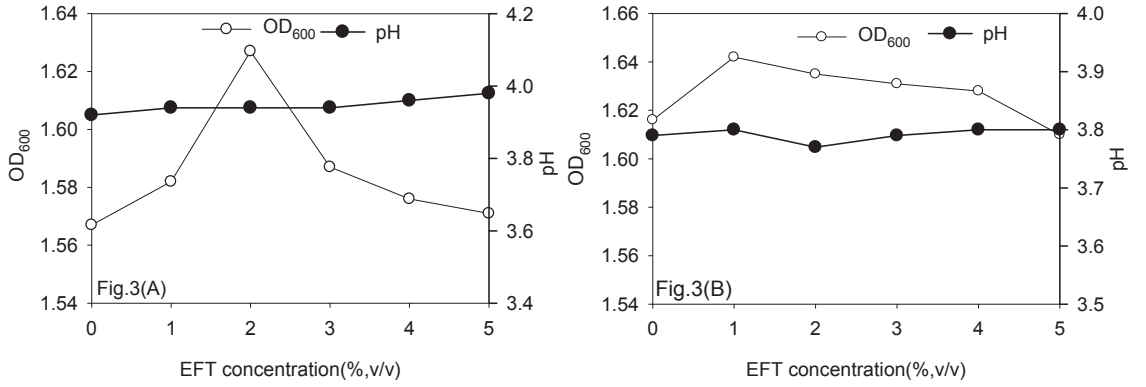
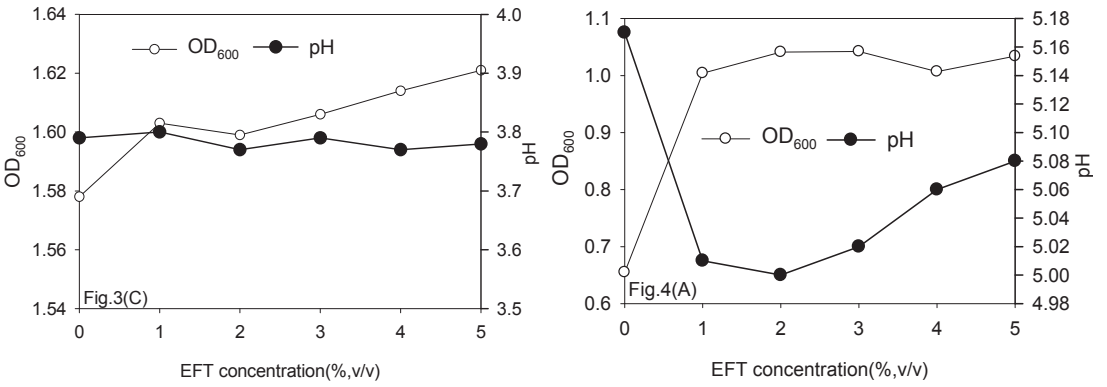



Fig3.Effect of EFT on growth of *Lactobacillus acidophilus LA06*,A,B and C mean incubation 12h,18h and 24h,respectively




*3.4. Effect of EFT on growth of L. acidophilus LA09*

Effect of different concentrations of EFT on the growth of *Lactobacillus acidophilus LA09* showed in Figure 4. With the increasing concentration of EFT at incubation 12h, OD value increased gradually from 0.655 at 0% EFT to 1.042 at 3%EFT, then decreased to 1.034 at 5% EFT. At incubation 18h, The OD value increased gradually from 0.914 at 0% EFT to 1.181 at 1% EFT, then decreased to 1.115 at 5% EFT. At incubation 24h, The OD value increased gradually from 1.027 at 0% EFT to 1.265 at 1% EFT, then decreased to 1.141 at 5% EFT. the pH of different culture mediums in contrast with the $OD_{600}$ has significant difference ranging from 5.01 to 5.17 (incubation time 12h), 4.79-5.09(incubation time 18h and 24h), which indicated that EFT on growth of *Lactobacillus acidophilus LA09* has an significant promotion, and the optimum concentration of EFT in MRS media was 1%.

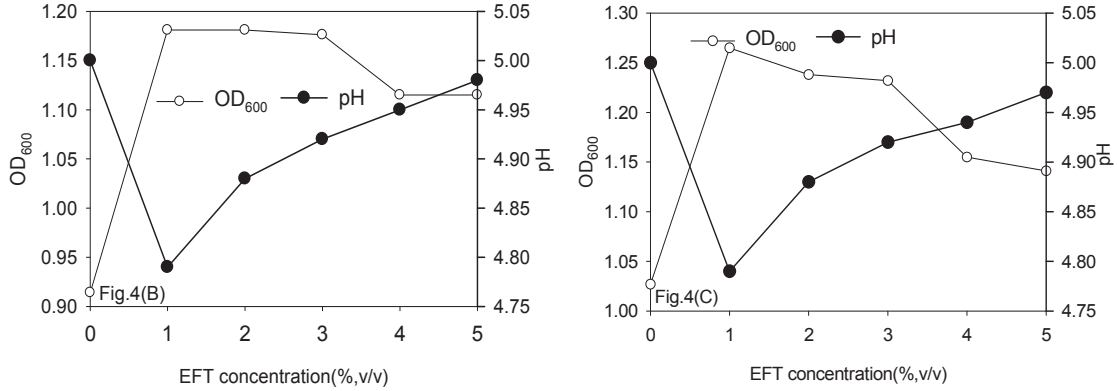



Fig4.Effect of EFT on growth of *Lactobacillus acidophilus LA09*,A,B and C mean incubation 12h,18h and 24h,respectively

## 4. Conclusions

Addition of EFT in MRS media has the significant promotion on growth of *Lactobacillus acidophilus LA09,* but has no obvious effect to *Lactobacillus acidophilus LA04, LA05* and *LA06*. The optimum concentration of EFT in MRS media was 1% for *Lactobacillus acidophilus LA09*.

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